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Response of a GH- and TSH-Secreting Pituitary Adenoma to a Somatostatin Analogue (SMS 201-995): Evidence that GH and TSH Coexist in the Same Cell and Secretory Granules

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Abstract. A 29-year-old male presented with acromegaly and hyperthyroidism and was found to be hypersecreting both GH and TSH. A somatostatin analogue, SMS 201-995, at doses of 50 and 100 μg s.c. 3 times a day produced an acute decrease in serum GH and TSH levels to less than 20% of basal concentrations. An increase in serum SMS 201-995 levels preceded the decline in serum GH and TSH levels. Partial resolution of signs and symptoms related to GH excess occurred and the patient developed normal serum thyroxine levels. These latter effects were maintained during the 3.5 months of SMS 201-995 therapy; however, pituitary adenoma size as judged by MRI was unchanged. Side effects of therapy were minimal and included transient abdominal pain, diarrhea and weight gain. Adenomatous pituitary tissue was surgically removed and placed in monolayer culture. It was observed that SMS 201-995 produced significant inhibition of GH and TSH release. Histology revealed a partly chromophobic, partly acidophilic adenoma containing GH and TSH. Electron microscopy showed a pituitary adenoma which appeared to consist of smaller cells resembling somatotrophs and larger cells exhibiting ultrastructural features of thyrotrophs. Immunoelectron microscopy localized the two biochemically distinct peptides in the same cell type, often in the same secretory granules. No morphologic abnormality, attributable to SMS 201-995 medication, was evident. Thus it can be concluded that pituitary adenomas can simultaneously secrete GH and TSH which produce acromegaly and hyperthyroidism. These bi-hormonal tumors may synthesize GH and TSH in the same cell type. Administration of SMS 201-995 can inhibit the secretion of both GH and TSH, causing significant clinical and biochemical improvement of acromegaly and hyperthyroidism. Suppression of GH and TSH release by SMS 201-995 is not necessarily followed by tumor shrinkage and morphologic abnormalities in the adenoma cells.

Human pituitary adenomas may excessively release more than one hormone [1]. These multipeptide secreting pituitary tumors may produce these hormones from different cell types in the tumor or one cell type may hypersecrete more than one hormone [2]. We recently evaluated a young male with classic acromegaly and markedly elevated growth hormone secretion who also had signs and laboratory findings compatible with TSH-induced hyperthyroidism. TSH-induced hyperthyroidism has been rarely seen in acromegaly [3, 4] and has been previously managed by radiation and surgical therapy. In light of reports that a somatostatin analogue (SMS 201-995) could decrease GH levels in acromegaly [5–7] and that TSH can be inhibited by somatostatin [8] we began therapy with the analogue prior to surgical removal of the tumor. Marked improvment in the acromegaly and resolution of the hyperthyroidism occurred within 1 month after initiation of somatostatin therapy. The direct effect of the somatostatin analogue on tumor secretion of GH and TSH were examined in cell cultures of the tumor. In addition morphologic studies of the tumor including immunohistochemistry, ultrastructural analysis and immunoelectron microscopy provided an opportunity to examine whether one or more cell types were responsible for the excessive secretion of two biochemically diverse pituitary peptide hormones.

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Materials and Methods

Clinical Presentation

A 29-year-old white male was referred for possible acromegaly. He stated that approximately 8 years ago he noted progressive enlargement of his fingers requiring three changes in ring

size. His foot size also enlarged from a size 10 to a 12EE. He noted a marked decrease in exercise tolerance and muscle strength and he perspired excessively. His wife noted cessation of breathing frequently during his sleep which was compatible with sleep apnea. The physical exam demonstrated prognathism, increased spaces between his teeth, an enlarged tongue and lips, frontal bossing, markedly enlarged hands and feet, and a goiter. He was 5 ft 11 in weighed 194 lb, and his initial sitting blood pressure was 140/90. During the following 3 months of our evaluation his blood pressure increased modestly. His visual field exam revealed a right temporal superior quadratic field defect. An MRI scan revealed a large homogenous pituitary mass which enveloped the left carotid artery in its cavernous portion and elevated the optic chiasm. Normal clinical chemistry levels were obtained for serum electrolytes, creatinine, calcium, total protein, CBC and ALT. Abnormal general laboratory exams included an elevated alkaline phosphatase (bone fraction) of 229 (normal < 105) and an increased serum phosphate of 5.9. His alkaline phosphatase remained elevated during somatostatin therapy and following pituitary surgery. His bone densitometry was normal. General endocrine evaluation revealed normal serum glucose levels during an oral glucose tolerance test and normal pituitary-adrenal function (urine and plasma cortisol and catecholamines as well as plasma ACTH levels). He had diminished plasma testosterone of 0.84 ng/ml (normal 3.5–10 ng/ml) whereas his serum LH was normal (3.9 mIu/ml) and his FSH was elevated at 13 mIu/ml (normal 4–10 mIu/ml). His serum thyroxine level was elevated (see table 1) and a radioactive iodine uptake and scan revealed thyroid enlargement and an elevated 6- and 24-hour uptake of 23 and 41%, respectively.

Study Protocol

Following informed consent he was admitted to the OSU Clinical Research Center. A TRH infusion test was performed by infusing 500 µg i.v. over 1 min and obtaining blood samples via an indwelling small gauge needle for TSH, PRL and GH at 0, 30, 60, 90, and 120 min. Bromocriptine, 5 mg. p.o., administration was followed by venous blood sampling at 0, 30, 60, 120 and 180 min for GH and TSH levels. Also hourly sampling for 24 h was performed via an indwelling needle with serum being frozen at –4 °C until analyzed for GH, PRL, TSH and SMS 201-995 serum concentrations. Twenty-four hour profiles were also performed on the following days when 50 and 100 µg of SMS 201-995 were administered subcutaneously every 8 h. Treatment with SMS 201-995 (100 µg t.i.d.) was then initiated for 3.5 months with blood samples for GH and TSH obtained at 2-week intervals. A 24-hour profile and a TRH stimulation test were repeated 3 months following the initiation of therapy.

Tumor Culture

SMS 201-995 was continued for 3.5 months until surgery when tumor tissue was removed via a transsphenoidal approach and transported asceptically to our tissue culture laboratory. Monolayer cultures were prepared as we have previously described [9]. This large tumor provided numerous cells (6×10^6) on which to perform multiple experiments in primary cultures. As control tissue we cultured four normal human pituitaries removed within 6 h of death and placed in culture as we have previously described [9].

Morphologic Studies

For light microscopy, pieces of tumor tissue were fixed in 10% buffered formalin and embedded in paraffin. Sections of 4–6 µm thickness were stained with hematoxylin-eosin and the PAS technique.

For immunohistochemistry, the avidin-biotin-peroxidase complex method was applied as described elsewhere [10]. Paraffin sections of 4–6 µm thickness were immunostained for GH, PRL, ACTH, TSH, FSH, LH and α-subunit [11, 12]. The source of antisera, dilutions, control procedures and other details have been reported in previous papers [13, 14]. Mirror sections were examined to reveal whether two hormones were present in the cytoplasm of the same cell or different cells.

For electron microscopy, small pieces of tumor tissue were fixed in 2.5% glutaraldehyde, osmicated, dehydrated in a series of graded ethanol, processed through propylene oxide and embedded in an Epon-Araldite mixture. Appropriate areas were selected on toluidine blue-stained semithin sections for the ultrastructural study. Ultrathin sections were stained with uranyl acetate and lead citrate and investigated with a Philips 410-LS electron microscope.

For the immunoelectron microscopic demonstration of adenohypophyseal hormones, the immunogold double labeling technique was applied [15–17]. Details of the procedure were described previously [14].

Assays

Serum PRL and GH were determined by a homologous radioimmunoassay (RIA) [18]. The intra- and interassay coefficients of variation were less than 10%, calculated using control samples assayed at three different points on the standard curve for both assays. Serum TSH levels were measured by RIA (Serono-Braintree, Mass.). The sensitivity of the assay is 0.1 µU/ml and the intra- and interassay coefficient of variation in our laboratory are 5 and 13%, respectively. Serum T_4 was determined by RIA (Serono-Braintree, Mass.) and the intra- and interassay coefficients of variation of 3 and 5%, respectively. T uptake was determined using an immunofluorescence technique (Abbott Labs, Chicago, Ill.) and the intra- and interassay coefficients of variation are less than 3%. SMS 201-995 levels were evaluated by double antibody RIA as previously described [19]. The assay has a sensitivity of 20 pg/ml and an intra- and interassay coefficient of variation of less than 12%.

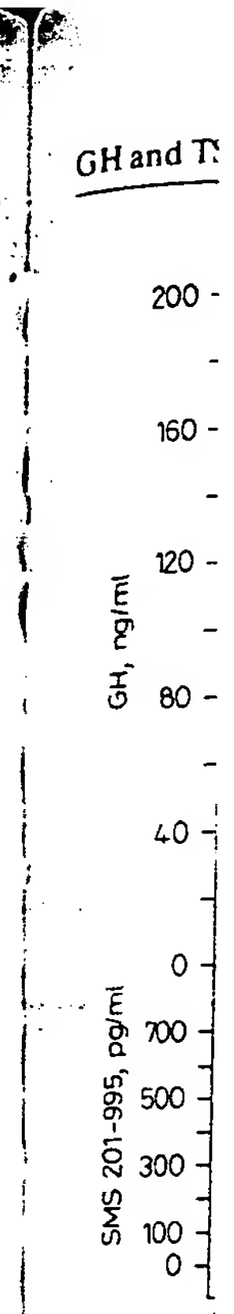
Statistical Methods

All data were entered into the data management CLINFO system located at the Clinical Research Center at Ohio State University. One-way analysis of variance with the Newman-Keuls aftertest was performed for endocrine data generated from the tissue culture studies. The 24-hour TSH and GH profile data were evaluated by Student's t tests. A p value of less than 0.05 was considered significant. The values are given as means ± SE.

Results

In vivo Studies

GH. The patient's 24-hour GH concentration prior to therapy was 132 ± 7 ng/ml with no nocturnal GH augmentation of secretion (fig. 1). An elevated somatomedin C level



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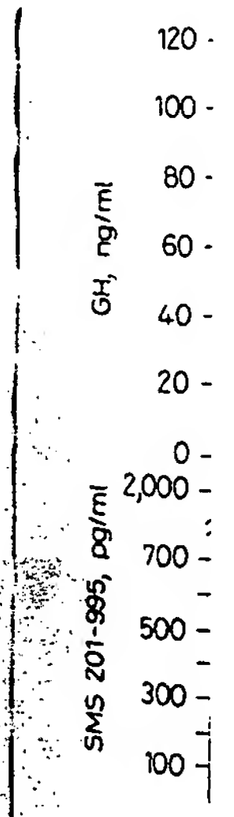

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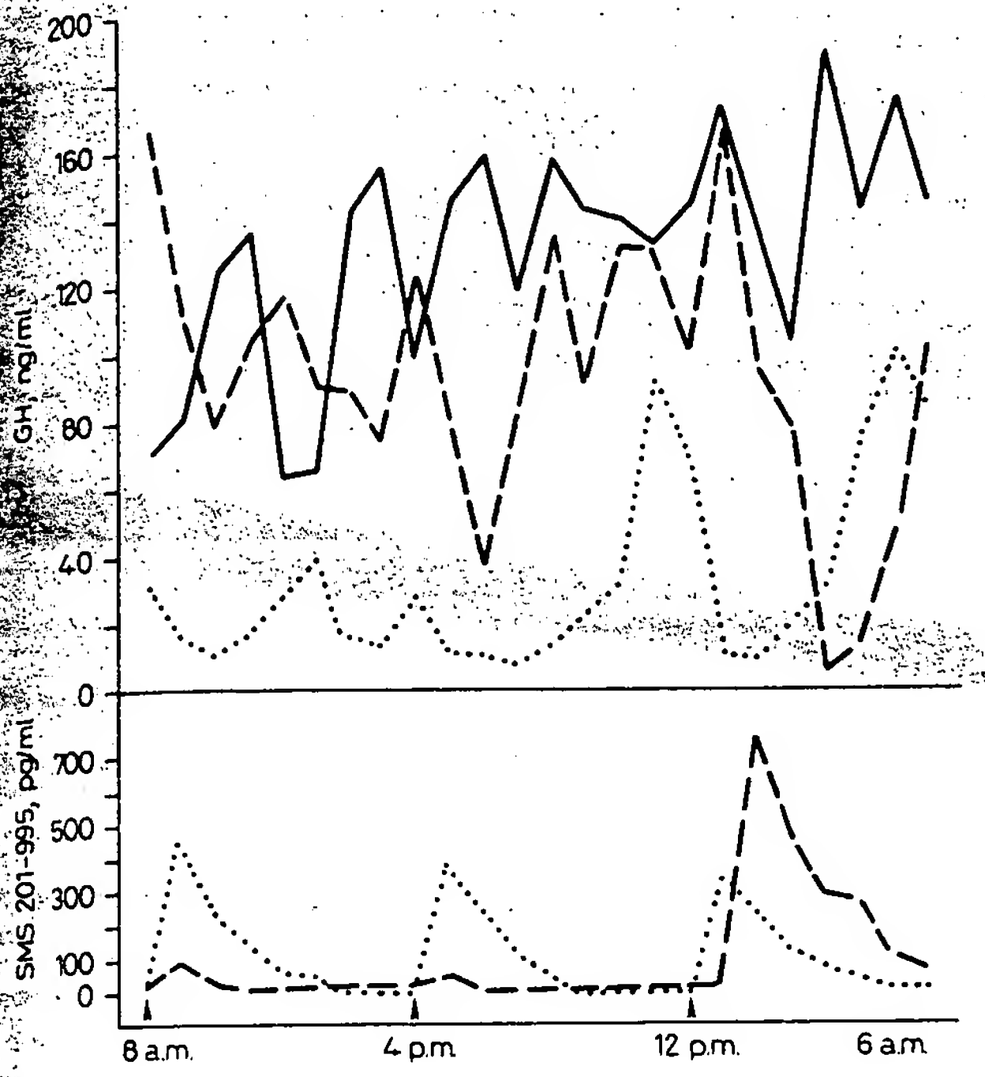

Fig. 1. Serum GH levels before (–), after 50 μg (——) and 100 μg (· · ·) of SMS 201-995 3 times a day (▲). Note that the dramatic decrease in serum GH levels is associated with a coincident peak in SMS 201-995 serum concentrations.

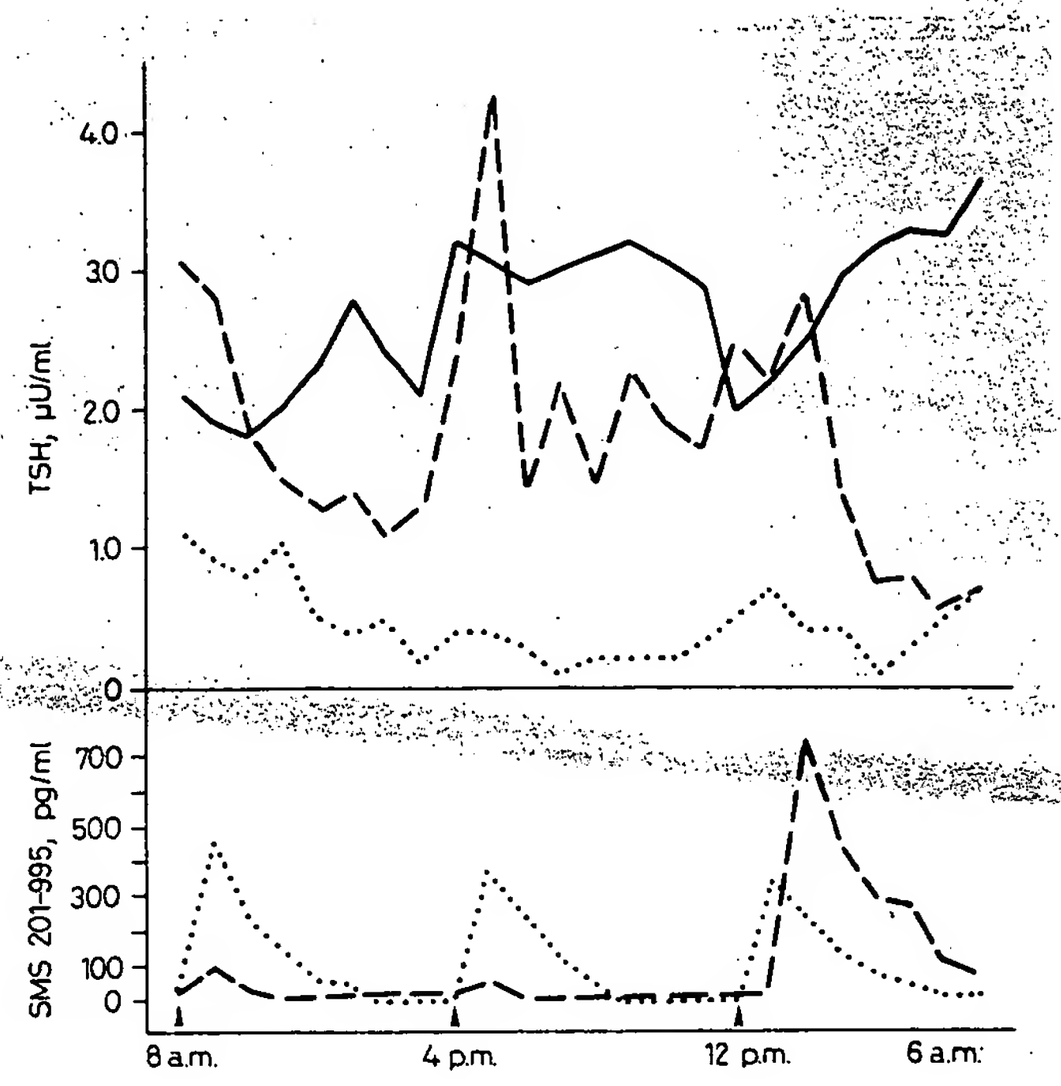

Fig. 3. Serum TSH levels before (–), and after 50 μg (——) and 100 μg (· · ·) of SMS 201-995 3 times a day (▲). Note that the dramatic decrease in serum TSH levels is associated with a coincident peak in SMS 201-995 serum concentrations.

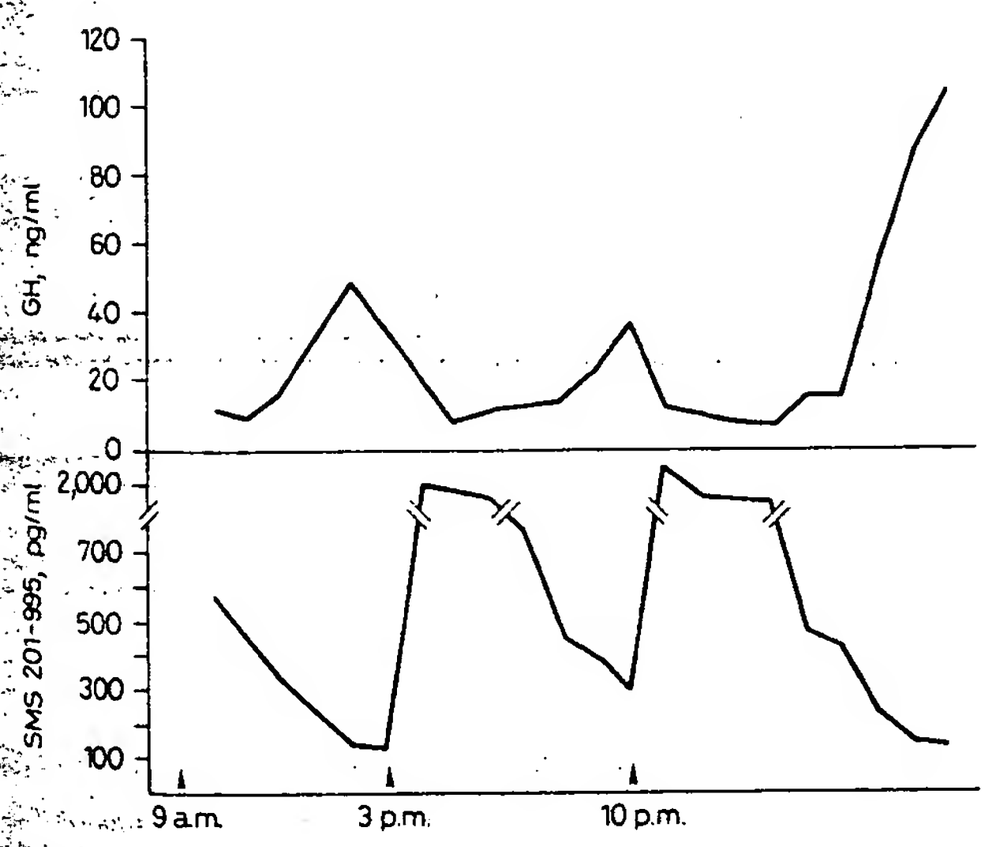

Fig. 2. Three months of 100 μg of SMS 201-995 3 times a day (▲) produced continued suppression of serum GH levels. Serum SMS 201-995 levels were approximately 4-fold higher than those initially measured (see fig. 1) following the same dose of the analogue.

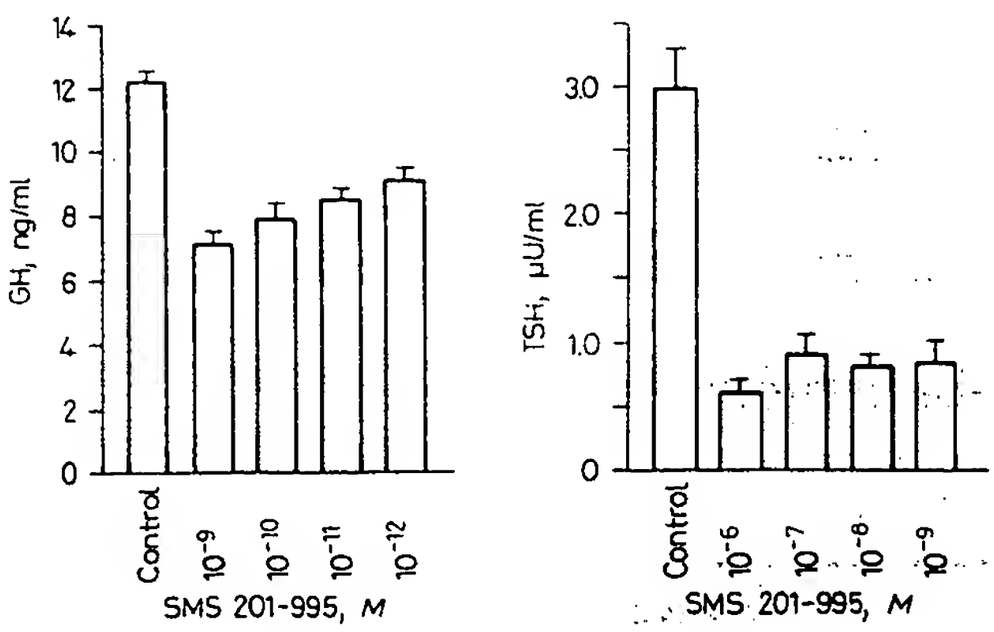

Fig. 4. GH and TSH secretion from monolayer cultures of the tumor was significantly ($p < 0.05$) inhibited by SMS 201-995. Concentrations of the analogue as low as $10^{-12}M$ produced significant ($p < 0.05$) inhibition of GH release and $10^{-9}M$ produced significant inhibition of TSH release within 60 min of its addition.

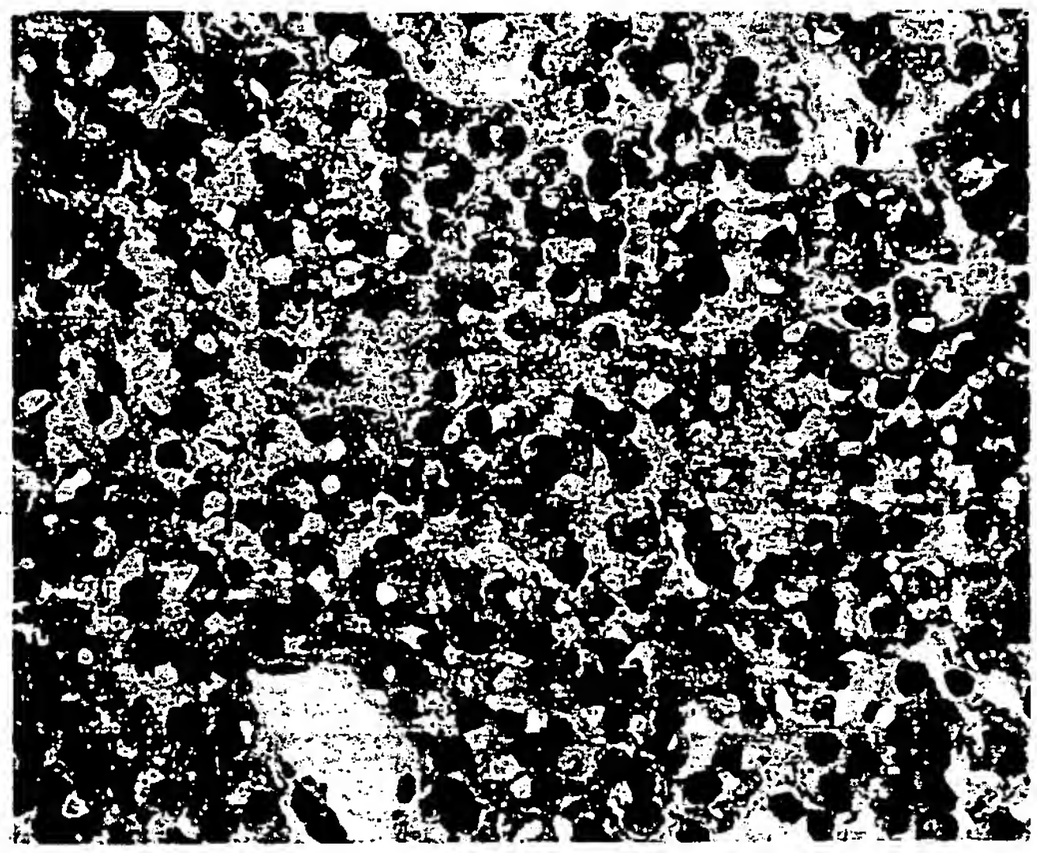

Fig. 5. Immunohistochemistry reveals the presence of growth hormone in the cytoplasm of many adenoma cells. Avidin-biotin-peroxidase complex technique for growth hormone. × 250.

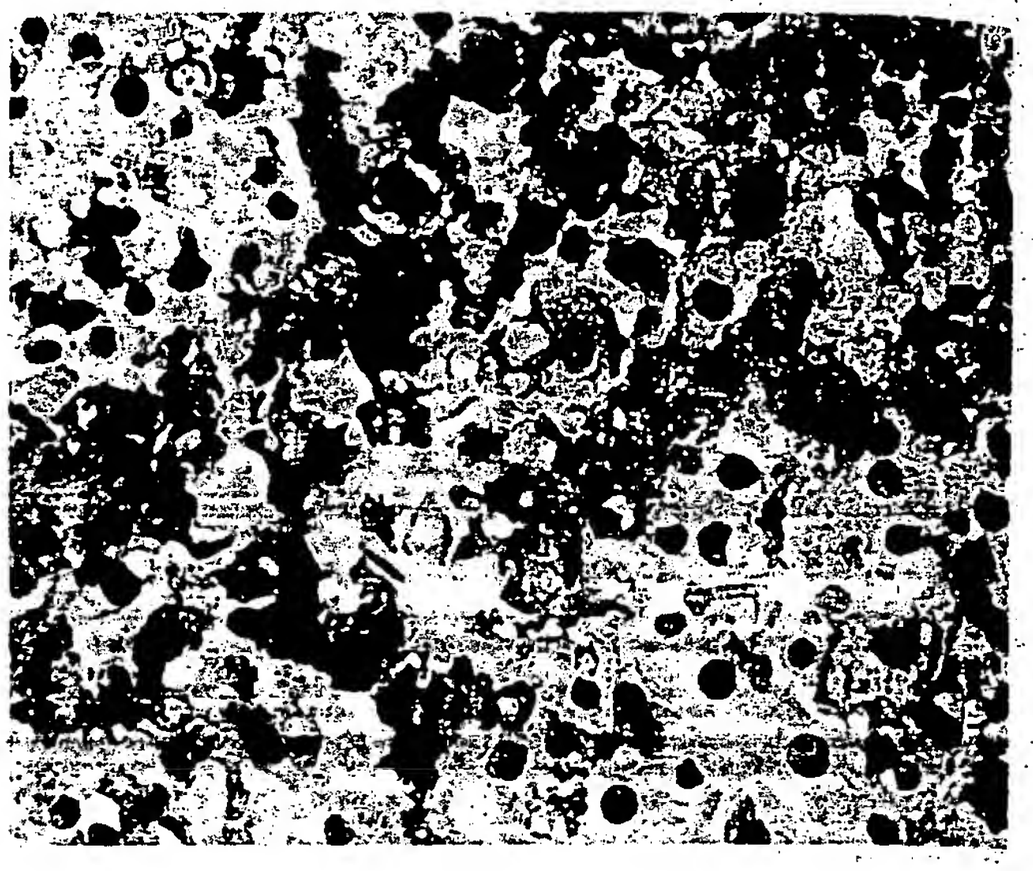

Fig. 6. Several adenoma cells contain immunoreactive TSH in their cytoplasm. Avidin-biotin-peroxidase complex technique for TSH. × 250.

of 488 ng/ml (normal 24–153 ng/ml) was also found. A 4-hour oral glucose tolerance test failed to completely inhibit his serum GH levels as the lowest GH level obtained was 27 ng/ml. TRH stimulation produced a paradoxical increase in serum GH from 39 to 238 ng/ml 1 h postinfusion. Bromocriptine stimualted GH release from 88 ng/ml to a peak of 218 ng/ml at 2 h following its administration.

When 50 μg of SMS 201-995 was administered every 8 h, serum GH levels fell immediately to a 24-hour level of 92 ± 9 ng/ml (pretreatment level was 132 ± 7 ng/ml (fig. 1). The following day 100 μg of SMS 201-995 every 8 h produced a further decrease in mean ± SE serum GH levels to 32 ± 6 ng/ml (fig. 1). The SMS 201-995 serum levels acquired during the initial 2 days of therapy demonstrated that peak levels of the analogue preceded the subsequent nadir in serum GH concentrations (fig. 1). Following 3 months of SMS 201-995 therapy (100 μg t.i.d.) the 24-hour GH levels remained at 37 ± 9 ng/ml (normal < 4 ng/ml) (fig. 2). Again the peak in serum SMS 201-995 levels preceded the nadir in serum GH levels. The serum SMS 201-995 levels after 3 months of therapy were 5 times higher than those noted on the initial day of 100 μg every 8 h of analogue therapy (fig. 1, 2).

PRL. The subject's 24-hour PRL level was minimally elevated prior to SMS 201-995 therapy at 15 ± 2 ng/ml (normal 9.3 ± 1) and did not change significantly following therapy (14 ± 3 and 13 ± 3 ng/ml) on 50 and 100 μg t.i.d. of SMS 201-995, respectively.

TSH and Thyroid Function

The patient's serum T_4 and FTI were in the thyrotoxic range (table I) but his TSH levels were not suppressed below 0.1 μU/ml, which is usually seen in hyperthyroidism. A TRH infusion failed to increase his TSH levels (basal 5.6 vs. 4.5 μU/ml peak TSH response). His 24-hour TSH level was 2.7 ± 0.1 μU/ml and did not exhibit normal diurnal variation (fig. 3). Following SMS 201-995 therapy the 24-hour TSH level decreased to 1.8 ± 0.2 and 0.5 ± 0.1 μU/ml after 50 and 100 μg of the analogue every 8 h (fig. 3). The decrease in TSH levels correlated with the increase in serum SMS 201-995 concentrations (fig. 3). Also a fall in TSH levels was associated with normalization of the serum T_4 and FTI values (table 1).

Culture Studies

Normal Human Pituitaries. SMS 201-995, 10^{-8}, 10^{-9} and $10^{-10}M$, added to four normal human pituitary monolayer cultures produced significant ($p < 0.05$) inhibition of GH release by 60 min in wells from only one human pituitary (control wells 44 ± 4 ng/ml, SMS 10^{-8}, 10^{-9}, and $10^{-10}M$ = 24 ± 2, 24 ± 3, and 27 ± 4 ng/ml, respectively). SMS 201-995 (10^{-6}, 10^{-7}, and $10^{-8}M$) did not significantly alter PRL release in any of these four normal pituitaries or significantly influence LH, FSH, or TSH release in a single pituitary that was studied [data not shown].

Adenomatous Monolayer Cultures

GH. The addition of SMS 201-995 to the cultures at doses between 10^{-6} and $10^{-12}M$ produced significant inhibition ($p < 0.05$) of GH secretion after 60 min of incubation (fig. 4). TRH 10^{-6} and $10^{-7}M$ produced a significant ($p < 0.05$) 2-fold increase in GH release 3 h after its addition to the wells and each dose of bromocriptine 10^{-6} and $10^{-8}M$ produced a significant ($p < 0.05$) 66% decrease in GH re-

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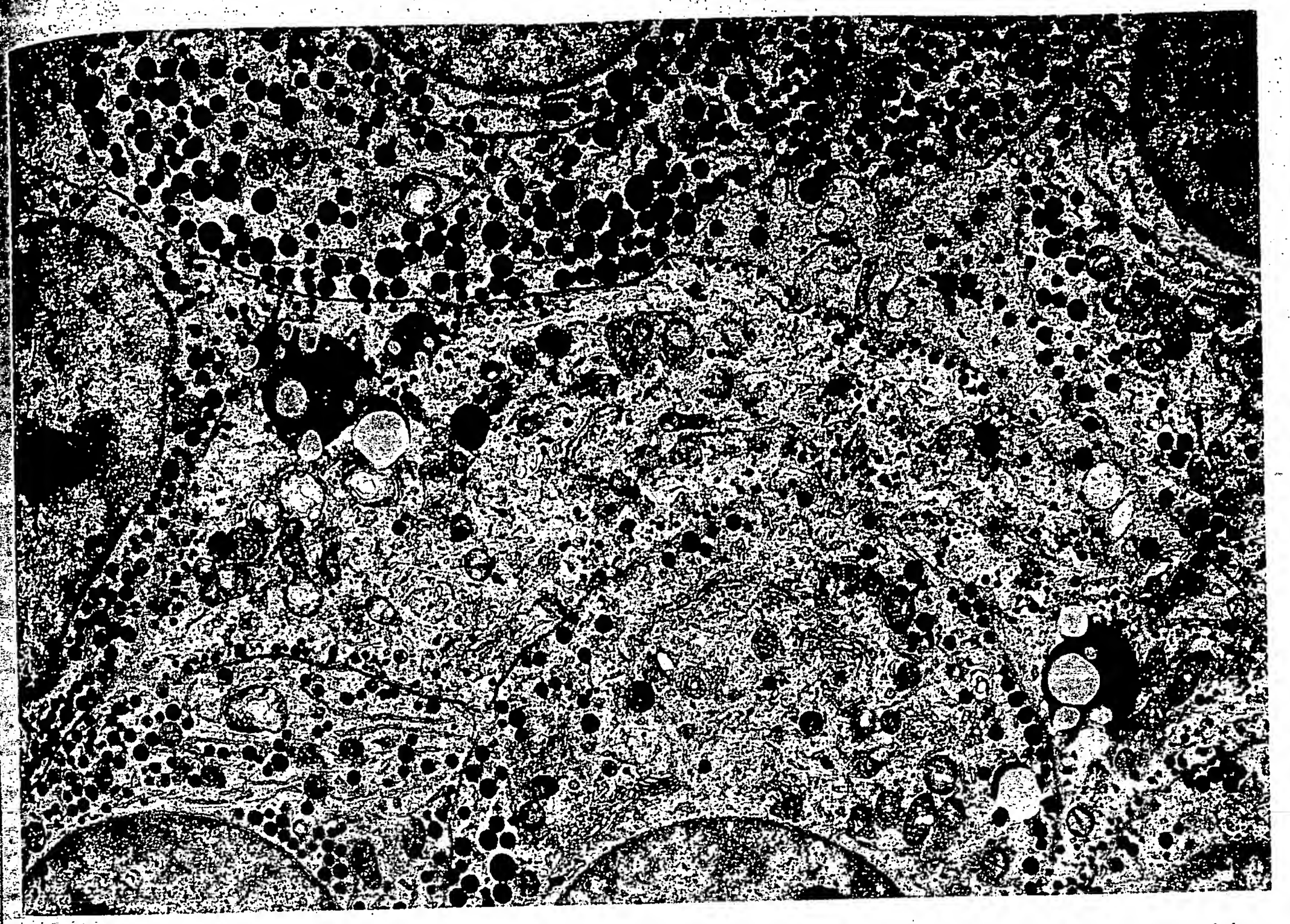

Fig. 7. Electron microscopy demonstrates adenoma cells exhibiting ultrastructural features similar to densely granulated growth hormone cells, whereas other adenoma cells (center) resemble thyrotrophs. Transmission electron microscopy. × 8,200.

lease by 1 h after its addition to the cultures [data not shown].

TSH and PRL. SMS 201-995, 10^{-6} to 10^{-9} *M*, produced significant ($p < 0.05$) inhibition of TSH-secretion from the tumor monolayer cultures by 60 min following its addition to the wells (fig. 4). Basal secretion of PRL from the cultures was extremely low, less than 1 ng/ml 24 h, and the addition of bromocriptine, TRH and SMS 201-995 did not significantly affect this low level of PRL release [data not shown].

Morphologic Studies

By histology, the tumor was a partly chromophobic, partly acidophilic adenoma of the pituitary exhibiting a diffuse pattern with some variation in cellularity, slight focal nuclear pleomorphism and occasional mitotic figures. In the cytoplasm of some adenoma cells, a few fine PAS-positive granules were noted. Immunohistochemistry revealed GH in the cytoplasma of many adenoma cells mainly in the cellular areas (fig. 5). Immunoreactive TSH was demonstrated in many large cells with abundant cytoplasm scattered throughout he tumor (fig. 6). Occasional adenoma cells exhibited immunopositivity for PRL. Immunostainings were negative for ACTH, FSH, LH and α-subunit. Consecutive sections showed the coexcistence of GH and TSH in he cytoplasm of some adenoma cells.

By electron microscopy, the adenoma appeared to be cellular and was found to consist of small, closely apposed cells (fig. 7). The nuclei were large, spherical or slightly oval with prominent nucleoli. The cytoplasm contained conspicuous lamellar endoplasmic reticulum membranes, moderately developed Golgi apparatus and numerous, mostly spherical, evenly electron-dense secretory granules measuring 150–400 nm, the majority measuring 250–300 nm. These cells were regarded as densely granuled somatotrophs. Several scattered adenoma cells were angular with long thin cytoplasmic processes. Based on their ultrastructural appearance, these cells seemed to represent a second cell type, which contained fairly well-developed membra-

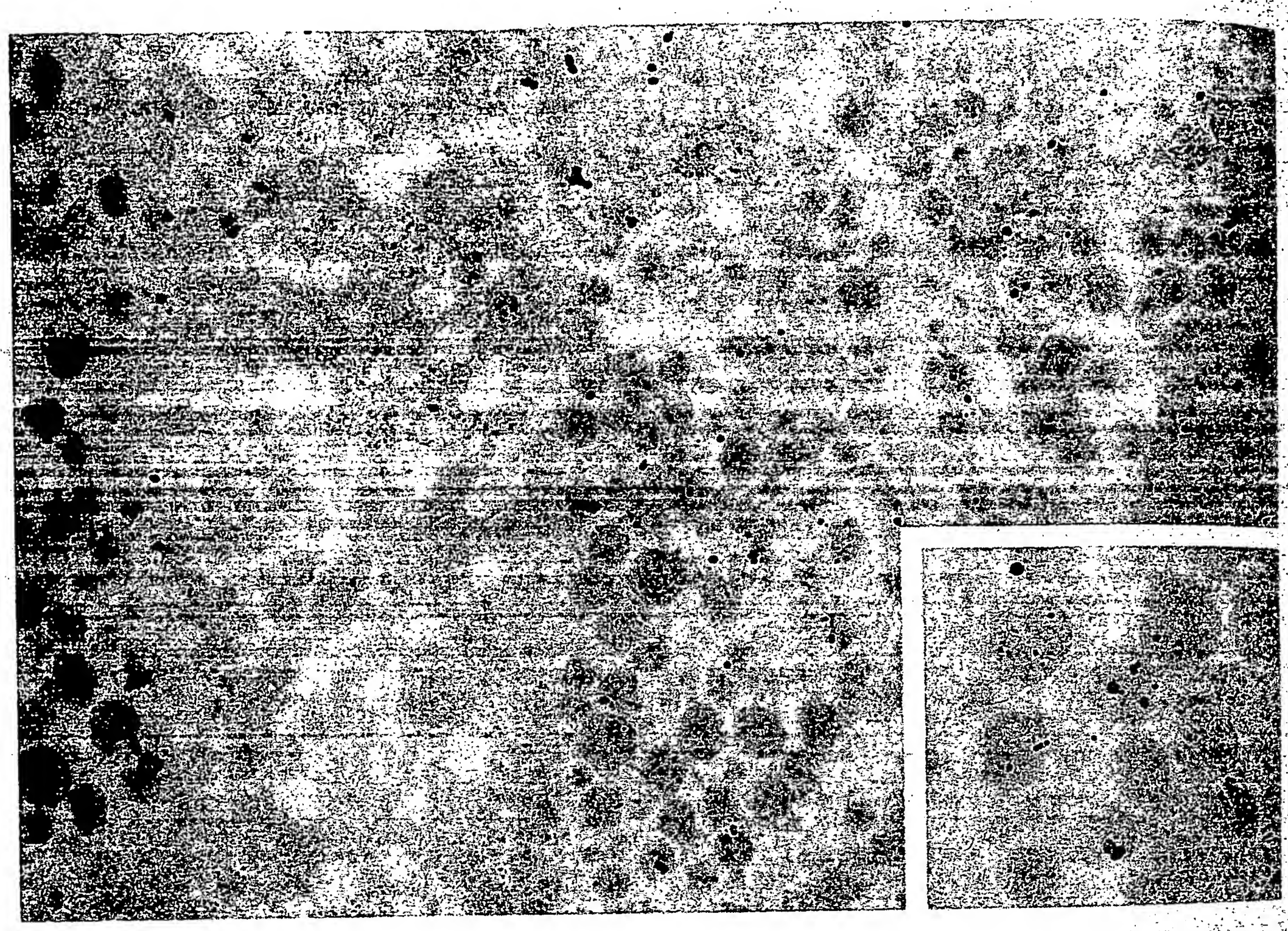

Fig. 8. Immunoelectron microscopy reveals a densely granulated bihormonal cell containing both growth hormone (40 μm gold particles) and TSH (15 μm gold particles) in the same and in different secretory granules. × 21,500 (inset × 36,600).

Table 1. Thyroid function tests before and during 3 months of SMS 201-995 treatment

	T_4 μU/100 ml	TU	FTI	TSH μU/ml
Before treatment	15.8	0.70	22.5	3.1
After treatment				
1 month	9.7	0.84	11.5	1.0
2 months	6.6	0.90	7.3	0.7
3 months	8.0	0.84	9.5	0.4
6 months postoperatively	10.7	0.80	13.4	1.5
Normal	3.5–11.0	0.7–1.25	3.5–11.0	0.2–5.5[a]

[a] Normal range if T_4 is normal; less than 0.1 μU/ml if T_4 or FTI is elevated.

nous organelles and sparse, spherical secretory granules measuring 100–200 nm lining up in a single row under the plasmalemma. The latter cells harbored large, compartmentalized lysosomal bodies, a characteristic ultrastructural feature of thyrotrophs [1]. No lactotrophs were identified in the electron-microscopic specimens available for study.

Immunoelectron microscopy confirmed the presence of GH and TSH in the adenoma cells (fig. 8). Some adenoma cells contained GH only, others TSH only, whereas others both GH and TSH. The two hormones were present in the same or in different secretory granules. No PRL, ACTH, FSH, LH or α-subunit could be demonstrated in the available specimens.

Clinical Response to Therapy

SMS 201-995. Following several weeks of 100 μg t.i.d. of SMS 201-995 therapy we observed a decrease in soft tissue swelling of the patient's hands, feet and face. His energy improved and he noted a decrease in his excessive perspiration. This clinical improvement was associated with a de-

crease in somatomedin C levels from pretherapy range of 488–768 ng/ml (normal 24–153 ng/ml) to a 6-month post-therapy value of 150 ng/ml. Side effects from the SMS 201-995 injections included abdominal pain, which was transient, and diarrhea which was only moderately bothersome. No significant affect on glucose tolerance was noted. A repeat MRI scan following 3.5 months of SMS 201-995 therapy did not reveal any significant decrease in the size of the tumor mass.

Postoperative Course

Following surgery a marked decrease in soft tissue mass of the patient's face, hands and feet was noted. His serum GH level remained at 26 ng/ml, which was similar to the preoperative GH level obtained while on SMS 201-995 injections. Six months following surgery his GH level was 22 ng/ml (normal < 5 ng/ml) and his somatomedin C level was 430 ng/ml (normal 24–153 ng/ml). His thyroid function studies obtained 6 months postoperatively revealed the return of minimal TSH-induced hyperthyroidism which was not associated with any detectable clinical symptoms or signs (table 1).

Discussion

We have demonstrated that injections of the somatostatin analogue, SMS 201-995, were able to significantly inhibit GH and TSH secretion from a pituitary macroadenoma. An acute reduction in GH and TSH levels to 24 and 12%, respectively, of pretreatment levels was noted within 2 days of initiating SMS 201-995 therapy. The degree of GH and TSH suppression correlated with SMS 201-995 serum levels and GH and TSH suppression was maintained throughout the course of therapy. Our findings with GH in this study were similar to those of several investigators who have previously reported on the efficacy of SMS 201-995 in the treatment of acromegaly [5–7]. The inhibiting influence of SMS 201-995 on GH and TSH secretion in vivo appeared to be at least partly directed at the pituitary as SMS 201-995 produced inhibition of GH and TSH secretion from the cultured pituitary tumor cells. This latter observation correlated with the previous observation of somatostatin receptors in pituitary adenomas of acromegalic patients [20, 21].

It has been previously demonstrated that SMS 201-995 can produce a decrease in the nocturnal rise in TSH release in normal man [8]. In addition Comi et al. [22] have recently shown that SMS 201-995 can significantly inhibit TSH release in patients with TSH-secreting pituitary adenomas and Wemeau et al. [23] in evaluating a patient similar to ours have also documented decrease in GH and TSH secretion with SMS 201-995. In our patient normalization of serum TSH levels by SMS 201-995 was associated with a return of serum thyroxine levels to normal prior to surgery.

Lowering of serum GH levels in this patient produced marked clinical improvement with decrease in tissue swelling and an increase in exercise tolerance. The side effects of SMS 201-995 therapy in this patient included diarrhea, and transient abdominal colic. The latter finding has previously been reported [6] and none of these side effects required a reduction in dose of the analogue. The suppression of serum GH and TSH levels was associated neither with any significant shrinkage of the adenoma nor with any microscopic abnormalities in the adenoma cells. This effect of SMS 201-995 to inhibit GH hormone secretion but not reduce pituitary tumor size has been previously reported [6].

Immunohistochemical, ultrastructural and immunoelectron-microscopic evaluation of the tumor produced evidence that the adenoma cells were excessively producing GH and TSH. Immunoelectron microscopy demonstrated that some adenoma cells contained GH only, others TSH only, whereas others contained both GH and TSH. It is noteworthy that GH and TSH were demonstrated by immunoelectron microscopy in cells regarded as either somatotrophs or thyrotrophs based on their ultrastructural appearance by conventional transmission electron microscopy. Thus the two allegedly different cell types showed no immunoelectron-microscopic difference.

Previous reports cite the coexistence and release of several biochemically distinct hormones from the same pituitary adenoma. Following administration of a secretagogue both hormones are released and it has been argued that these tumors may contain distinct hormonal cell types but they have similar receptors for the stimuli being investigated. Alternatively these biochemically different hormones may be secreted by a single cell type.

Our data supports the latter view in that we have demonstrated that both GH and TSH were found in the same pituitary cell type and even were found to coexist in the same secretory granule. Recently Beck-Peccoz et al. [24] demonstrated the presence of secretory granules positive for GH and a α-subunit in somatotroph adenoma cells and McComb et al. [25] reported a pituitary adenoma containing α-subunit and PRL in the cytoplasm of a single cell type. Hence evidence is accumulating that some pituitary adenomas which secrete biochemically diverse peptides may arise in a common progenitor.

Thus it can be concluded that pituitary adenomas can simultaneously secrete GH and TSH, which produces acromegaly and hyperthyroidism. Also these bihormonal tumors may synthesize GH and TSH in the same cell type. Administration of SMS 201-995 can inhibit the secretion of both GH and TSH causing significant clinical and biochemical improvement of the acromegaly and hyperthyroidism. Finally, suppression of GH and TSH release by SMS 201-995 is not necessarily followed by tumor shrinkage and morphologic abnormalities in the adenoma cells.

Acknowledgement

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